Coordinate Regulation of G Protein Signaling *via* Dynamic Interactions of Receptor and GAP

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***Short Abstract* — G protein signaling modules are ubiquitous throughout eukaryotes and convey information with diverse patterns of response speed, sensitivity and amplification. While they contain only four proteins –- receptor, heterotrimeric G protein, GTPase-activating protein (GAP) and effector –- they display complex signal output behaviors whose underlying mechanisms have resisted analysis. We developed a kinetic model of a prototypical G protein module and determined its parameter set (reaction rate constants) by fitting to data derived from extensive *in vitro* steady-state GTPase measurements. We used the model and parameters to investigate the signal output behaviors of this system and their biochemical mechanisms.**

Signal output from receptor-G protein-effector modules is a dynamic function of the nucleotide exchange activity of the receptor, the GTPase-accelerating activity of GTPase-activating proteins (GAPs) and their interactions. GAPs may inhibit steady-state signaling, but may also accelerate deactivation upon removal of stimulus without significantly inhibiting output when receptor is active [1]. Further, some effectors (phospholipase C-β, p115RhoGEF, *e.g.*) are themselves GAPs, and it is unclear how such effectors can be stimulated by G proteins at the same time as they accelerate G protein deactivation. The multiple combinations of protein-protein associations and interacting regulatory effects that allow such complex behaviors in this system do not permit the usual simplifying assumptions of traditional enzyme kinetics and are uniquely subject to systems-level analysis.

We developed a kinetic model for G protein signaling that permits analysis of both independent and interactive G protein binding and regulation by receptor and GAP [2]. We evaluated parameters of the model (all forward and reverse rate constants) by global least-squares fitting to a diverse set of steady-state GTPase measurements in a reconstituted m1 muscarinic receptor-G_q-phospholipase C-β1 module in which GTPase activities were varied by ~10^4-fold by manipulating the concentrations of GAP, GTP, GDP and receptor agonist. We provide multiple tests to validate and statistically qualify the fitted parameter set, which is consistent with results from the few previous pre-steady-state kinetic measurements that are available [3,4]. Results indicate that: (1) GAP potentiates the GDP/GTP exchange catalyst activity of the receptor, an effect never before reported; (2) exchange activity of the receptor is biased toward replacement of GDP by GTP; (3) receptor and GAP bind G protein simultaneously but with negative cooperativity when G protein is bound to either GTP or GDP, which promotes rapid GAP binding and dissociation; (4) GAP indirectly stabilizes the continuous binding of receptor to G protein throughout the GTPase cycle during steady-state GTP hydrolysis, thus further enhancing receptor activity; and (5) receptor accelerates GDP/GTP exchange primarily by opening an otherwise closed nucleotide binding site on the G protein and has minimal effect on equilibrium affinity ($K_{assoc}=k_{assoc}/k_{dissoc}$) of G protein for nucleotide.

Model-based simulation explains how GAP activity can accelerate deactivation >10-fold upon removal of agonist but still allow high signal output while agonist is present. Analysis of GTPase flux through distinct reaction pathways and consequent accumulation of specific GTPase cycle intermediates indicates that, in the presence of a GAP, receptor remains bound to G protein throughout the GTPase cycle, which obviates slow second-order receptor-G protein binding that is rate-limiting in the absence of GAP. In contrast, GAP binds primarily during the GTP-bound phase of the GTPase cycle and is often in rapid exchange with the receptor-G protein complex. The analysis explains these behaviors and relates them to the specific regulatory phenomena described above. The work generally demonstrates the applicability of appropriately data-constrained system-level analysis to signaling networks of this scale.

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